

Supplementary data

Metabolic engineering of the carotenoid biosynthetic pathway toward a specific and sensitive inorganic mercury biosensor

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Table. S1 Bacterial strains and plasmids used in this study

Strains and plasmids	Genotypes or description	Reference
<i>E. coli</i> strains		
TOP10	F ⁻ Φ80 <i>lacZ</i> ΔM15 Δ <i>lacX74 recA1</i>	Invitrogen
Rosetta(DE3)	F ⁻ <i>ompT hsdSB(r_B⁻ m_B⁻) gal dcm lacY1</i> (DE3)	Novagen
R/Hg-EBI	Rosetta(DE3) harboring Hg(II) biosensing construct pPmer-CrtEBI with red carotenoid lycopene as pigment signal	This study
R/Hg-EBIY	Rosetta(DE3) harboring Hg(II) biosensing construct pPmer-CrtEBIY with an orange carotenoid β-carotene as pigment signal	This study
R/Hg-EBIY-4	Rosetta(DE3) harboring Hg(II) biosensing construct pPmer-CrtEBIY-4 with an orange carotenoid β-carotene as pigment signal	This study
Plasmids		
pET-21a	Amp ^R , T7 promoter, <i>lac</i> operator	Novagen
pPmer	pET-21a derivative containing <i>merR</i> and <i>Pmer</i> divergent promoter region cloned into <i>Bgl</i> III and <i>Xba</i> I sites	1
pET-crtEBI	pET-21a derivative containing the lycopene biosynthetic gene cluster (a tricistronic <i>crtEBI</i> unit)	This study
pET-crtEBIY	pET-21a derivative containing the β-carotene biosynthetic gene cluster <i>crtEBIY</i> (a tricistronic <i>crtEBI</i> unit fused with <i>crtY</i> under the control of independent <i>Pmer</i> promoter)	This study
pPmer-crtEBI	pET-crtEBI derivative containing the lycopene biosynthetic module under the control of the Hg(II) sensory module	This study
pPmer-crtEBIY	pET-crtEBIY derivative containing the β-carotene biosynthetic module under the control of the Hg(II) sensory module	This study
pPmer-crtEBIY-4	pPmer-crtEBIY derivative containing <i>crtE</i> , <i>crtB</i> , <i>crtI</i> , and <i>crtY</i> under the control of independent <i>Pmer</i> promoter	This study

TACGCGGCCAGCAGGGTTGGCAGCTGCAGACCCTGCTGCGTGAAGAACAGGGCGCGCTGCCGATCACTCTGTCTGGCAACGCGGATGC
 GTTCTGGCAGCAGCGTCCGCTGGCATGCAGCGGCCCTGCGTGCCGGCCTGTCCACCCGACCACCGGCTACTCCCTGCCGCTGGCCGTTG
 CCGTGGCTGATCGTCTGTCTGCGCTGGATGTTTCACCTCCGCTTCCATCCACCACGCGATCACCCACTTCGCTCGTGAACGTTGGCAGCA
 GCAGGGTCTTCCGATGCTGAACCGTATGCTGTTCTGGCAGGTCGGCTGATAGCCGTTGGCGTGTGATGCAGCGCTTCTATGGTCTG
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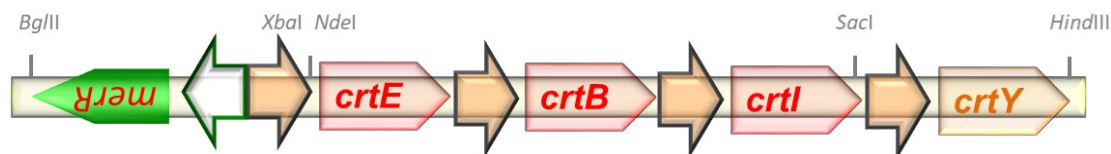


Fig. S1 The pigment-based Hg(II) biosensing constructs used in this study.

The lycopene biosynthetic gene cluster is comprised of *crtE* (909 bp), *crtB* (930 bp), and *crtI* (1479 bp). The β -carotene biosynthetic gene cluster is comprised of *crtE* (909 bp), *crtB* (930 bp), *crtI* (1479 bp), and *crtY* (1149 bp). These pigment synthetic genes are initially from *Pantoea ananatis* (GenBank: D90087.2) and are regulated by the metalloregulator MerR.

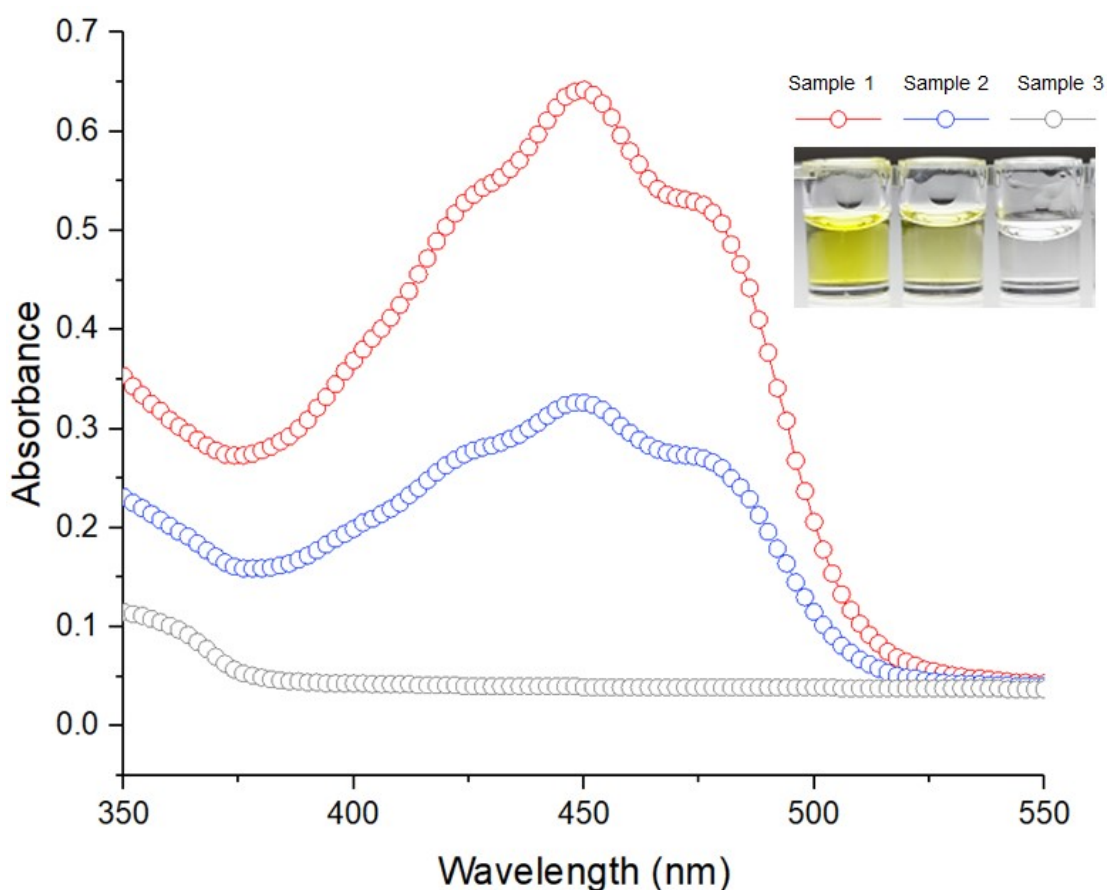


Fig. S2 The visible absorbance curves of commercial β -carotene.

The capsule containing β -carotene was purchased from Puritan's Pride (New York, USA). Contents of one capsule were extracted with 2 mL ethanol. The upper ethanol

extract (sample 1), the double dilution (sample 2), and the ethanol extract of the cell pellets of host Rosetta(DE3) (sample 3) were scanned at wavelengths of 350-550 nm at intervals of 2 nm.

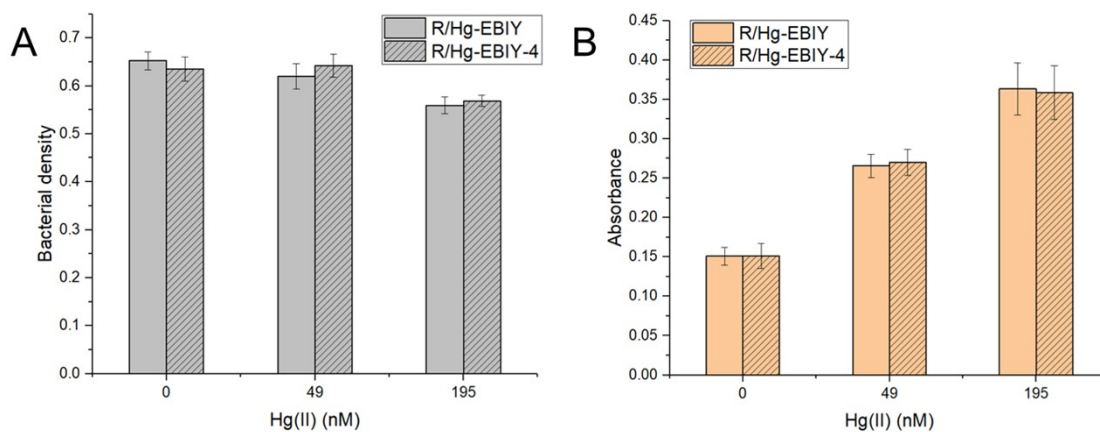


Fig. S3 Comparison of the response to Hg(II) in two bacterial biosensors.

(A) Bacterial densities of R/Hg-EBIY and R/Hg-EBIY-4 after exposure to different concentrations of Hg(II). (B) The response intensity of R/Hg-EBIY and R/Hg-EBIY-4.

The values shown are mean \pm SD ($n = 3$).

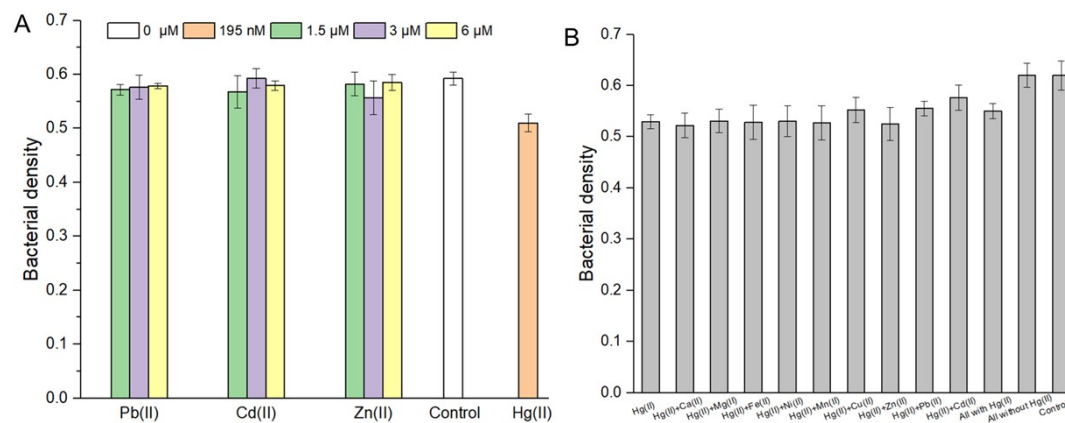


Fig. S4 Bacterial densities of R/Hg-EBIY exposed to various metal ions after incubation at 37 °C for three hours.

(A) Bacterial densities after exposure to Pb(II), Cd(II), Zn(II), and Hg(II) at different concentrations. (B) Bacterial densities after exposure to 195 nM Hg(II) in combination with 6 μ M divalent metal ions. Data shown are mean \pm SD ($n = 3$).

References

1. C. Y. Hui, Y. Guo, H. Li, Y. T. Chen and J. Yi, *Frontiers in microbiology*, 2022, **13**, 846524.